

Eupera troglobia sp. nov.: the first troglobitic bivalve from the Americas (Mollusca, Bivalvia, Sphaeriidae)

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Abstract

Eupera troglobia sp. nov. is the first fully described troglobitic bivalve discovered in the Americas, and possibly the second in the world. The troglobitic designation is based on the lack of pigmentation, reduction in size and shell thickness, and large and few offspring. Unlike its relatives that live in the roots of the water hyacinth, this new species is attached to the walls of the caves with a byssus. The anatomical study shows some peculiarities in comparison with the congeners, especially the simplicity of the foot, the siphons and the gut. This discovery is a new contribution to the efforts for the conservation of subterranean habitats in Brazil.

Keywords

Anatomy, cavernicolous life, conservation, Mollusca, new species, Systematics

Introduction

Troglobitic animals are restricted to subterranean habitats and therefore unable to establish viable populations in surface habitats. Such organisms are adapted to living under stressful conditions, such as lack of light and scarcity of food. As for mollusks, the typical troglobitic mollusk usually exhibits the following characteristics: (1) absent or reduced pigmentation, (2) absent or reduced eyes, (3) some reduction in shell size (reduction in size compared to their epigeal relatives) (Simone, in press). These characteristics have

been idealized using the most common cave-dwelling taxon – Gastropoda. In the Americas, only gastropods have been classified as troglobites. Especially in South America, most of them are freshwater gastropods, but some terrestrial gastropods are also known (Simone and Moracchioli 1994; Simone 2012, 2018, 2019; Simone and Salvador 2021).

The only other class of mollusks that occurs outside the ocean is the bivalves (Bivalvia). The occurrence of bivalves in subterranean habitats is sparsely documented. There are few reports of external species being transported to or living in caves, such as some species from the genera *Pisidium* Pfeiffer, 1821 (Grego et al. 2019) and *Euglesa* Jenyns, 1832 (Turbanov et al. 2016) (both Sphaeriidae). Recently, an interesting bivalve species was described for Germany (*P. interstitialis* Boessneck, Groh & Richling, 2020), which has an exclusively interstitial lifestyle and thus lives outside open waters (Groh et al. 2020). However, exclusively subterranean species are rare in this group. The only undoubtedly obligate cave bivalve belongs to the dreissenid genus *Congeria* Partsch, 1835, which occurs mainly in southeastern Europe (Bilandžija et al. 2013, 2021; Chong et al. 2016). Furthermore, the small bivalves of the genera *Pisidium* and *Euglesa* could also be cave-bound species, although this “status” certainly deserves further studies to confirm or refute it. Four species of *Pisidium* have been observed only in caves: one from Turkey and three from caves in the Caucasus Mountains in Abkhazia (Bole and Velkovrh 1986). In addition, three species of *Euglesa* have also been found only in caves, two from Georgia (in the region of Imereti and Abkhazia) and one from Russia and Georgia (Turbanov et al. 2016). It is interesting to note that the rarity of bivalves in caves may be due to their greatly reduced mobility combined with their general inability to tolerate hypoxic habitats (Culver 2012). Therefore, it is unlikely that the rarity of bivalves living only in caves is due to the lack of potential surface ancestors. Surprisingly, small bivalves that can be classified as troglobites were collected from Casa de Pedra cave, Lagoa da Confusão municipality, Tocantins, central region of Brazil.

The examination of this material revealed that it belongs to the sphaerid genus *Eupera* Bourguignat, 1854. The genus is relatively widespread throughout South America and is normally found in the roots of water hyacinth [*Eichhornia crassipes* (Mart.) Solms – Pontederiaceae], an aquatic plant that floats in calm freshwater environments. A total of 11 valid species of *Eupera* occur in South America east of the Andes (Simone 2006), all of which have characteristic shell pigmentation with dark brown spots in a beige ground color (Mansur and Veitenheimer 1975).

Bivalves have eyes in only a few lineages, the Sphaeriidae are not among them, so the absence of eyes in *Eupera* cannot be considered. However, the collected specimen has the other characteristics to be classified as a troglobite: Absence of pigment, fragile shell, and small size. All of these characteristics are described in detail below.

The first records of specimens of the cave clam described here appeared in a 2006 technical report of the National Center for Cave Exploration and Conservation (Centro Nacional de Pesquisa e Conservação de Cavernas – **CECAV**) (Silva 2006). In that report, a faunal survey of Casa de Pedra Cave was presented, focusing on invertebrates (Figs 33–35). Although the author presented some photographs of the clam, little attention was paid to this species, which was thought to have been carried into the cave by the water. In 2010, one of the authors of this paper (RLF) accessed the report and

considered the possibility that this species might be a troglobite due to its depigmentation. He searched for sampled specimens but was unable to find any in the collection where the remaining specimens were deposited (ISLA/UFLA), suggesting that specimens were not collected on this occasion. In 2021, a team visited the cave specifically to search for such shells. Although the cave was partially flooded (see Habitat and Ecological Notes section), the researchers were able to find specimens that are described here.

This paper addresses the official description of the first fully described troglobitic mollusk from the Americas, which may be the second mollusk group with cave-bound representatives, apart from the dreissenid genus *Congeria* (See in Discussion the other sphaeriids mentioned above). The study includes detailed anatomical and conchological investigations as a basis for comparison with other congeneric species.

Material and methods

The material was received for taxonomical and anatomical study already fixed in 70% ethanol, with no previous narcotization method. They were mainly deposited in the MZSP collection, with paratypes selected for other indicated institutions. The dissection procedures and terminology are standard (Simone et al. 2015), with the specimens dissected under dissecting stereomicroscope, immersed in the fixative. Digital photos of the dissecting steps were obtained by connected digital camera. SEM examination was also standard, with shells covered by gold; due to the fragility of the shells, some of them were broke during this process. The anatomical drawings were obtained with the aid of a camera lucida connected to the microscope. Each drawing (as well as the description) is a synopsis of the examination of several specimens.

The following abbreviations are used in the anatomical descriptions and figures

aa	anterior adductor muscle;	ci	ciliary gill connection;	if	mantle border inner fold;
ae	possible aesthete scar;	cv	cerebrovisceral connective;	in	intestine;
al	anterior lateral hinge tooth;	dd	duct to digestive diverticula;	ip	inner hemipalp;
an	anus;	dg	digestive diverticula (gland);	is	incurrent siphon;
ap	genital aperture;	em	embryos inside visceral brood pouch;	ki	kidney;
ar	anterior pedal retractor muscle;	es	esophagus;	li	ligament;
as	anterior hinge socket;	ex	excurrent siphon;	mb	mantle border;
au	auricle;	fb	foot base;	mf	mantle border middle fold;
bf	byssal furrow;	fg	gill food groove;	mo	mouth;
by	byssus;	ft	foot;	nv	nerve;
ca	cardinal hinge teeth;	hf	hinge mantle fold;	od	outer demibranch;
cc	cerebral commissure;	id	inner demibranch;	of	mantle border outer fold;
ce	cerebral ganglion;			op	outer hemipalp;

pa	posterior adductor muscle;	pr	posterior pedal retrac- tor muscle;	un	fusion between man- tle lobes;
pc	pericardium;	ps	posterior hinge socket;	ur	nephropore;
pe	periostracum;	rt	rectum;	ve	ventricle;
pg	pedal ganglia;	sh	shell;	vg	visceral ganglia;
pl	posterior lateral hinge tooth;	ss	style sac;	yo	young specimen in- side marsupium of inner demibranch.
pm	pallial muscle;	st	stomach;		
pn	pallial line;	su	suspensory membrane;		
pp	palp;	sy	statocyst;		
		um	umbonal region;		

Additional abbreviations:

ISLA/UFLA	Coleção de invertebrados Subterrâneos de Lavras – Universidade Federal de Lavras;
MNRJ	Museu Nacional da Universidade Federal do Rio de Janeiro;
MZSP	Museu de Zoologia da Universidade de São Paulo, Brazil;
USNM	National Museum of Natural History, Smithsonian Institution, Washington DC, USA.

Additional abbreviations:

LV	left valve;	spm	specimen(s) in alcohol;
RV	right valve;	W	width.
sh	shell(s);		

Taxonomy

Family Sphaeriidae

Genus *Eupera* Bourguignat, 1854

Type species. *Pisidium moquinianum* Bourguignat, 1854, Monotypy; = *Eupera bahiensis* (Spix in A. J. Wagner, 1827).

Eupera troglobia sp. nov.

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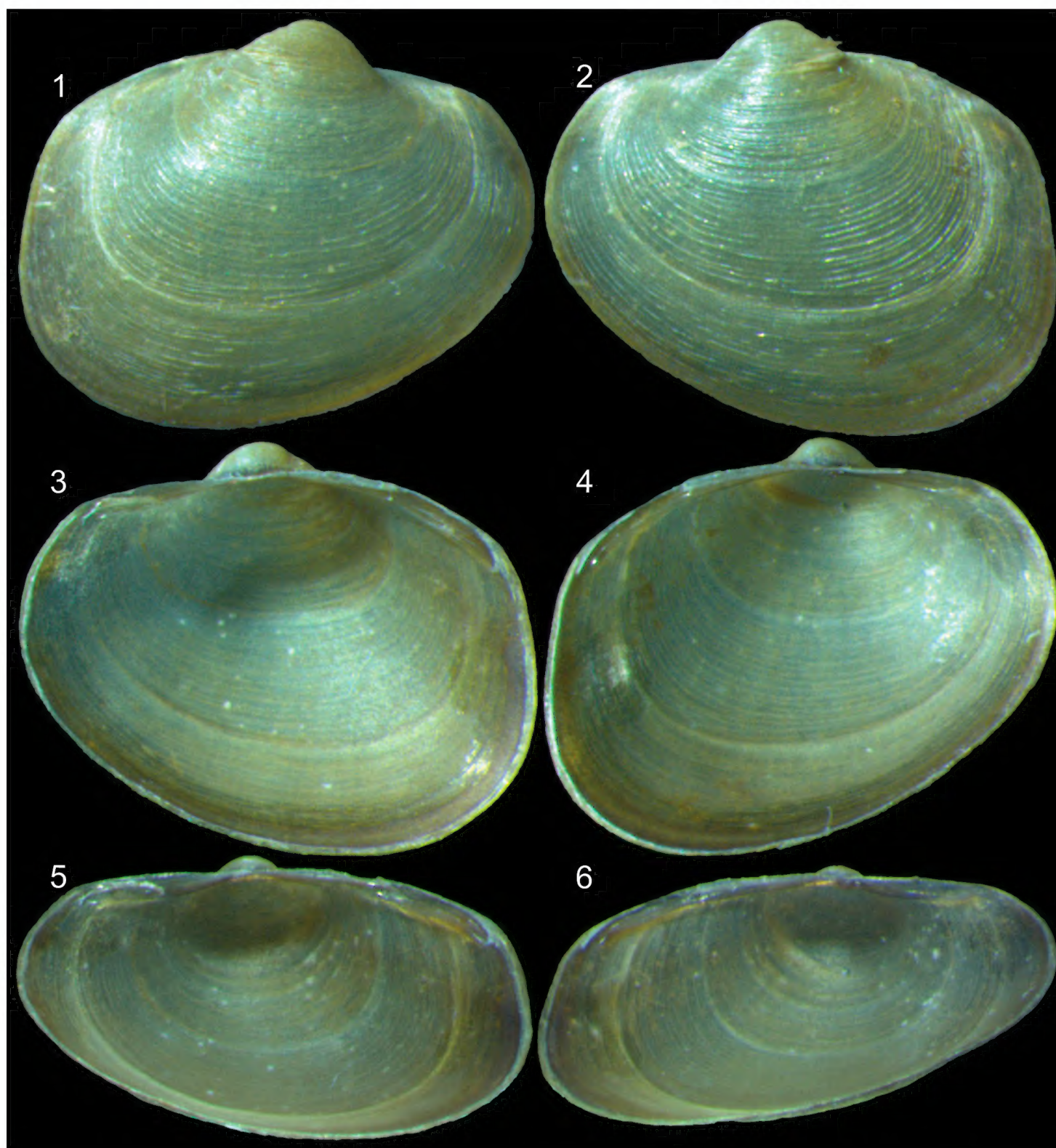
Figs 1–30

Material examined. Holotype. MZSP 155717. **Paratypes** MZSP 155716, 12 specimens, MNRJ 23647, 1 specimen, USNM, 1 specimen, all from type locality.

Type locality. BRAZIL. Tocantins; Lagoa da Confusão, Casa da Pedra cave, 10°49'28.4"S, 49°37'16.5"W [Ferreira col., 3.viii.2021].

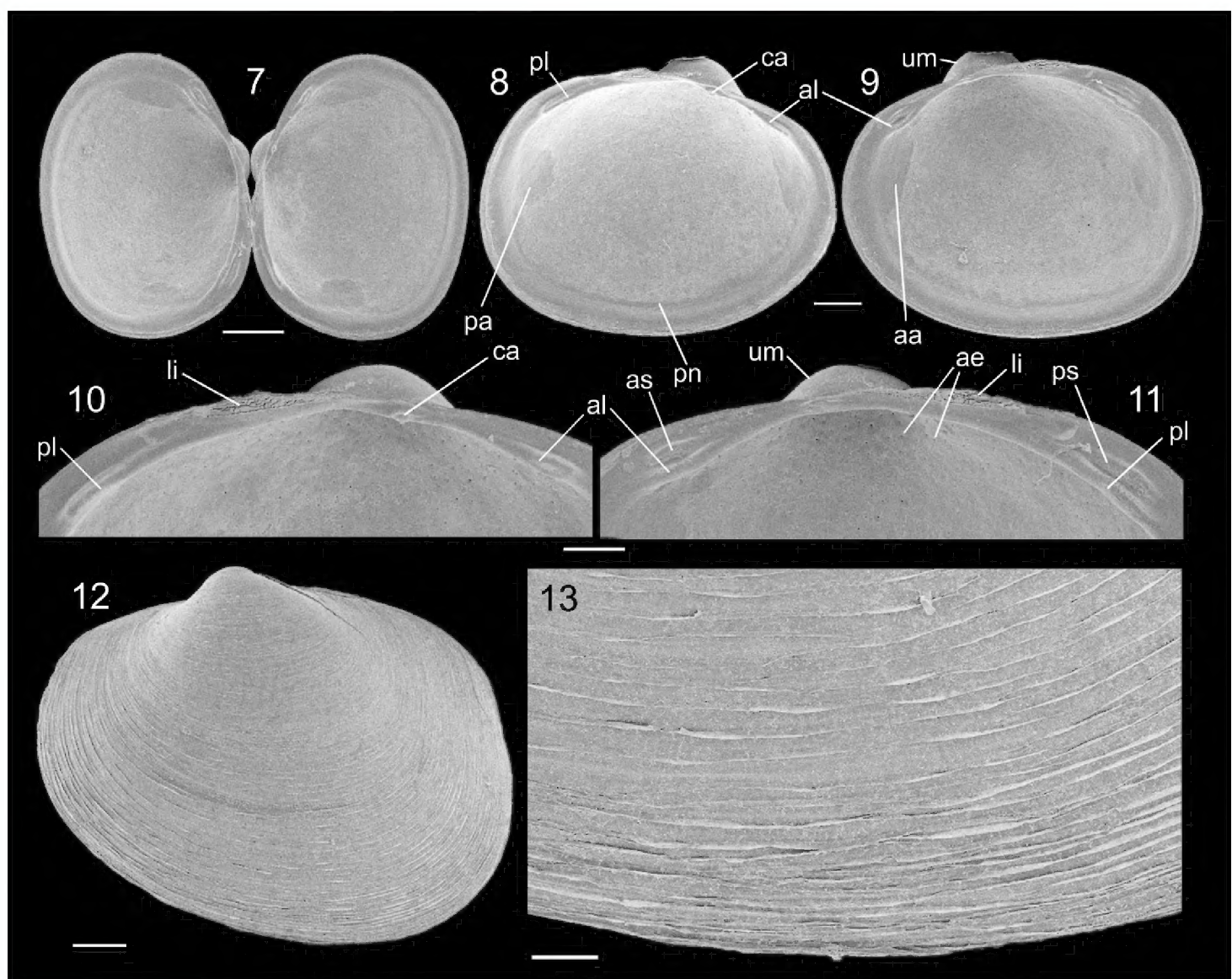
Diagnosis. Adult size ~4.5 mm. Lacking pigmentation in shell and soft parts. Shell very fragile, translucent, light yellow.

Description. Shell (Figs 1–18). Adult shell ~4.5 mm. Equivalve; height ~80% of length; width ~60% of length. Walls thin, fragile, translucent. Anterior edge rounded, smaller than posterior edge; ventral edge rounded in medium specimens (Figs 7–9) to slightly ascendent in larger specimens (Figs 1–4, 12); posterior edge almost straight in



Figures 1–6. *Eupera troglobia* holotype MZSP 155717 shell (L 4.3 mm), right valve in left column, left valve in right column **1** outer right view **2** outer left view **3** inner left view **4** inner right view **5** inner left-slightly ventral view **6** inner right-slightly ventral view.

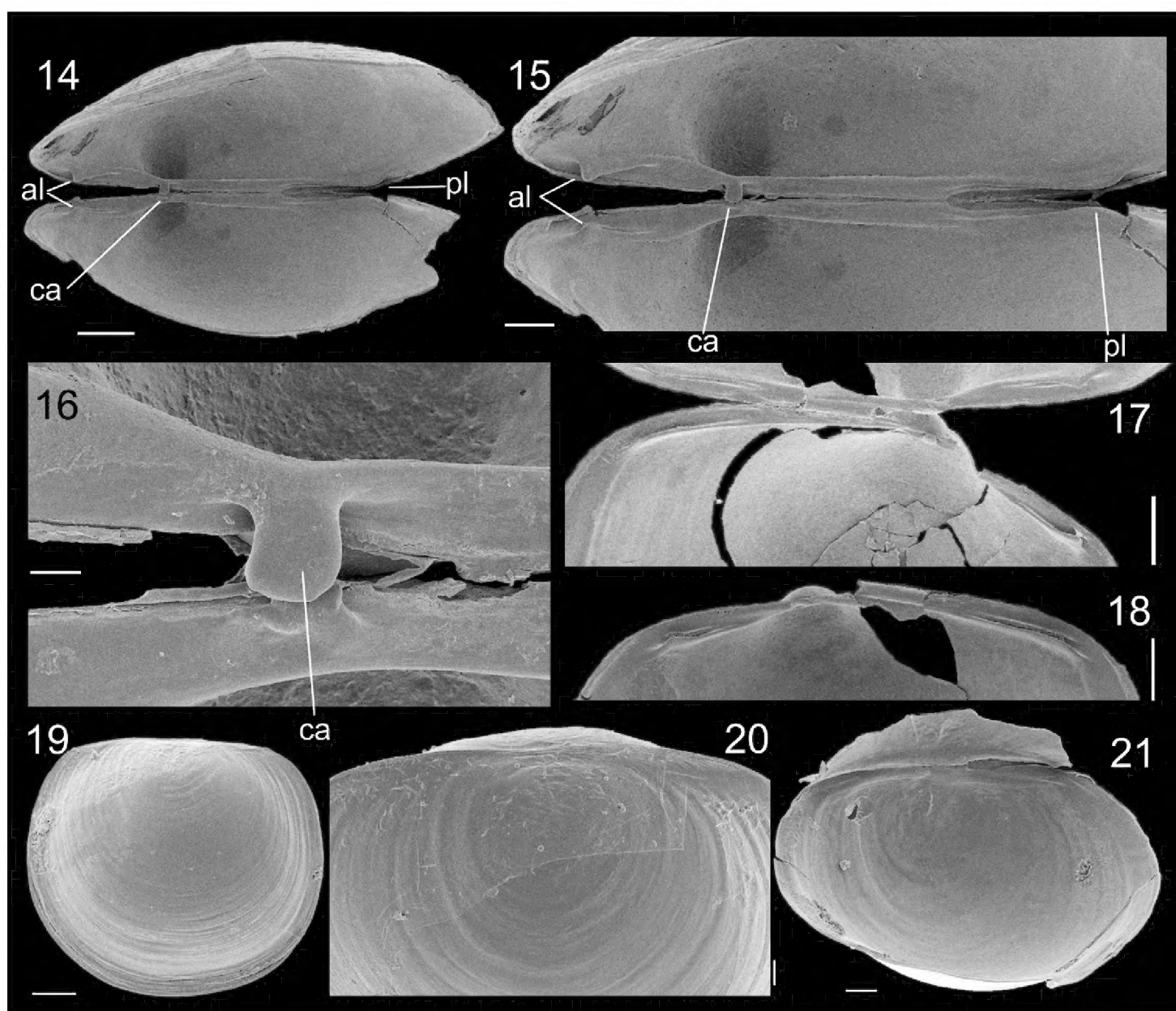
its middle level; dorsal edge weakly convex, almost straight. Color light yellow to light greenish yellow (Figs 1–6). Outer surface opaque. Sculpture of uniform concentric growth lines (Figs 1, 2, 12, 13); ~15 per mm; each line alternating in height along its length (Fig. 13), but mostly 4–5 times taller than wide; interspaces ~10 times wider than each line. Growth lines usually continuous from anterior up to posterior hinge region (Figs 1, 2, 12). Umbo (um) slightly prosogyrate, central, protruding ~10% height beyond hinge level (Figs 1–4, 8–9), occupying ~20% of dorsal edge. Hinge with small, blunt cardinal tooth (ca) in LV, ~1.5 times longer than tall, tip rounded (Figs 14–16), shallow correspondent socket in RV (Fig. 16); anterior (al) and posterior (pl) lateral teeth relatively equidistant from cardinal tooth (Figs 4–6, 7–11, 14–18), similar to each other, in both valves; located in anterior and posterior ends of hinge edge, in blunt angle preceding anterior and posterior slopes; each lateral tooth ~4-times longer than wide, parallel to hinge edge; anterior pair of tooth usually with anterior small beak (Figs 14–15: al, 17–18); both lateral teeth of LV slightly more ventral than



Figures 7–13. *Eupera troglobia* shell SEM images of paratypes 153866 **7** specimen #7, both valves connected, opened ~120°, ventral view **8** #7, left valve, inner left view **9** #7, right valve, inner left view **10** #7, left valve, detail of hinge region, inner left view **11** #7, right valve, detail of hinge region, inner right view **12** specimen #8, left valve, outer left view **13** same, detail of surface on middle region of ventral edge. Scale bars: 500 µm (**7**, **12**), 300 µm (**8**), 200 µm (**10**, **13**).

lateral teeth of RV, encasing ventrally to them; both lateral teeth of RV with narrow socket lying dorsally for counterparts of LV (Figs 5, 9, 11, 18). Inner surface glossy; scar of anterior adductor muscle (aa) occupying ~5% of entire inner valve surface, ~twice taller than wide, elliptic, slightly larger than scar of posterior adductor muscle (pa) (Figs 3–4, 7–9). Pallial line continuous, simple, connecting both adductor muscle scars; relatively broad; located along ventral edge ~15% of total height distant from it. Inner surface possessing minute pits, possibly of aesthetes (Figs 10–11: ae).

Main muscle system (Figs 22, 24, 27). Anterior adductor muscle (aa) with elliptic transverse section, dorso-ventral height of slightly twice anterior-posterior width; located close to blunt angulation between dorsal and anterior shell edges. Posterior



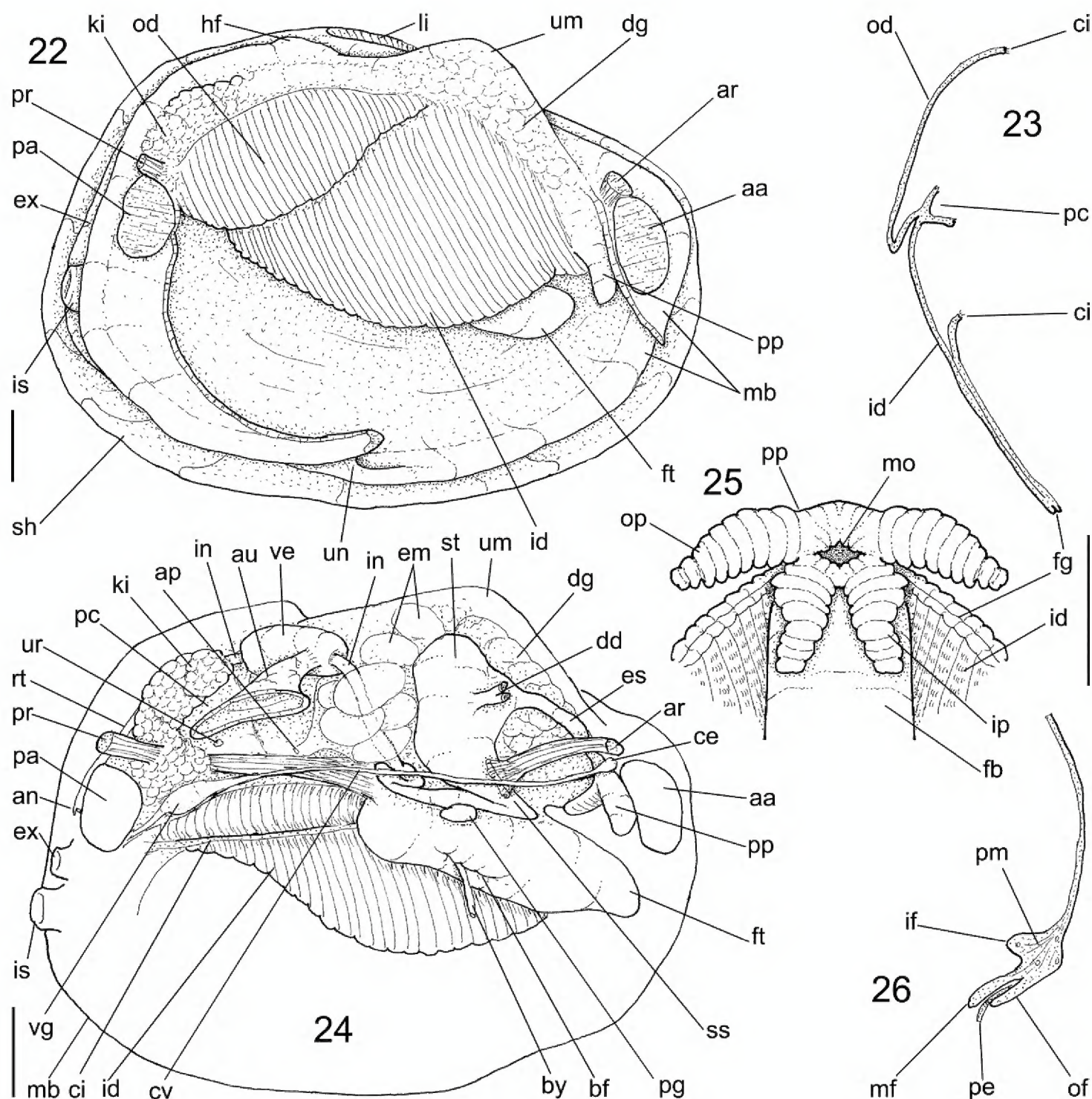
Figures 14–21. *Eupera troglobia* shell SEM images of paratypes 153866 **14** specimen #8 (part damaged), valves opened ~40°, ventral view **15** same, higher magnification **16** same, higher magnification, region of cardinal tooth **17** specimen #9 (part damaged), valves opened ~60°, ventral view, mainly showing hinge of left valve **18** same, right valve **19** shell of young specimen extracted from gill's marsupium, left valve, outer left view **20** same, detail of umbo showing prodissococonch in its center, left-slightly dorsal view **21** another intra marsupial specimen (part damaged), right valve, inner ventral-slightly left view, focus on hinge, part of left valve still attached by ligament. Scale bars: 500 µm (**14, 17, 18**), 300 µm (**15**), 100 µm (**19**), 50 µm (**16, 21**), 30 µm (**20**).

adductor muscle (pa) slightly smaller and located slightly more ventrally than anterior muscle. Pair of anterior pedal retractor muscles (ar) originated just dorsal to anterior adductor muscle in elliptic area equivalent to ~15% of that of anterior adductor muscle; running towards posterior relatively narrow, along ~20% of shell length, attached to adjacent visceral integument; insertion splaying in antero-dorsal foot base. No detectable protractor pedal muscle. Pair of posterior pedal retractor muscles (pr) originating similarly as anterior pedal muscle, but dorsally to posterior adductor muscle; running narrow anteriorly along ~50% of shell length, as central base to local visceral mass and attached to adjacent visceral integument; insertion splaying in postero-dorsal side of pedal base.

Foot (Fig. 24: ft). Cylindric, ~3-times longer than wide in contracted condition; ~half projected anteriorly beyond its base. Posterior region ventrally bulged, rounded. Anterior end bluntly tapering. Byssal furrow (bf) narrow, occupying ~1/4 of middle region of ventral foot surface. Byssus (by) as single, narrow, yellow thread.

Mantle (Figs 22, 26, 27). Mantle lobe thin, translucent, thickened only in edges. Colorless. Edges of both lobes fused with each other in region of anterior adductor muscle, and in region posterior to middle level of ventral edge; fusion provided by inner fold. Mantle edge in non-fused region (Fig. 26) with narrow, flattened outer (of) and middle (mf) folds, no papilla or special structures detectable; inner fold (if) located in inner base of middle fold, with ~half of remaining folds height, as wide as tall. Pallial musculature (pm) thin, present in base of three folds. Fusion between posterior half of mantle edges (un) simple. Incurrent siphon (Fig. 27: is) simple, cylindric, walls weakly muscular; distal edges simple, lacking papillae; length in retracted condition ~5% of shell length, ~twice longer than wide. Excurrent siphon (ex) similar to, but ~30% smaller than incurrent siphon; preserved inverted in several specimens. Siphonal musculature immersed in local mantel edges, lacking detectable bundles, neither producing pallial sinus in shell. Gill suspensory membrane (su) connected by cilia in posterior end of gill, membrane-like separating completely incurrent from excurrent chambers (Fig. 27).

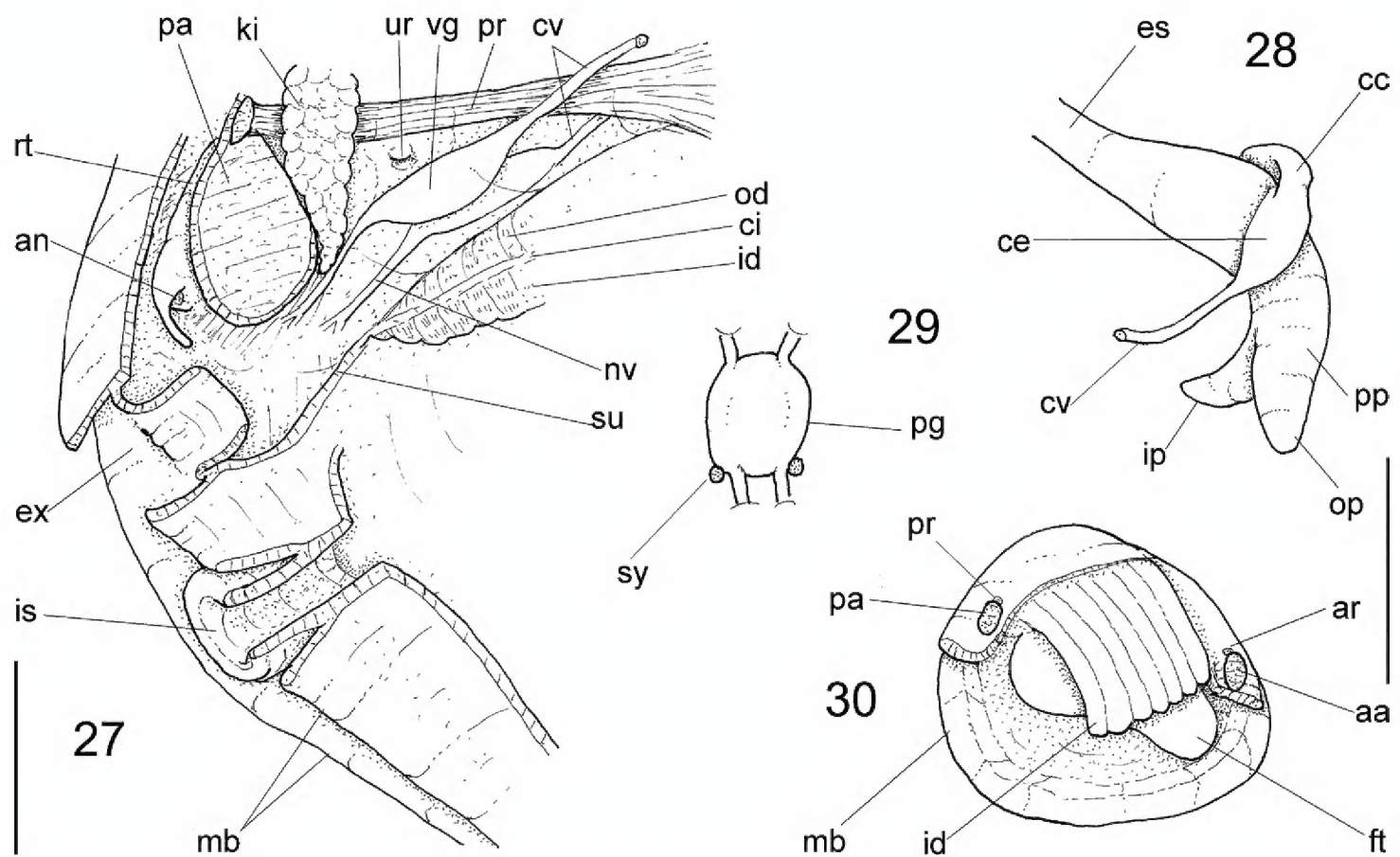
Pallial cavity (Fig. 22). Outer demibranchs (od) with ~1/4 of shell height in its middle region; tapering gradually towards anterior, up to middle level of inner demibranch dorsal edge; tapering subtly towards posterior; lamellae very narrow, with ventral curve covering small region of inner demibranch dorsal edge (Fig. 23: od); dorsal connection with visceral mass via cilia (ci). Inner demibranchs (di) wide, area ~double as outer demibranchs; anterior region slightly wider than half of shell height, gradually tapering towards posterior up to certain distance from posterior adductor muscle; transversely folded; descendent lamella (Fig. 23: id) simple, very narrow, free from ascendent lamella; ascendent lamella with ~70% of descendent lamella length; narrow food groove (fg) in inner demibranch ventral edge; inner demibranch connections with visceral mass and its counterpart (in posterior half – Fig. 24) via cilia (ci). Inner demibranch serving as marsupium of ~6–8 young specimens (Fig. 31: yo), detailed below. Pair of palps (Figs 24, 25: pp) small (~half of anterior adductor muscle area), located just posterior to anterior adductor muscle; outer hemipalps (op) ~3-times longer than



Figures 22–26. *Eupera troglobia* anatomical drawings **22** whole right view, right valve and part of right mantle lobe removed **23** gill, transverse section in its middle level **24** whole right view, right gill removed, visceral structures seen as in situ if region was transparent, peripheral structures with only topology indicated **25** palp region, ventral view, hemipalps slightly deflected **26** mantle edge, transverse section in middle level of ventral edge. Scale bars: 0.5 mm.

wide, 8–10 strong transverse folds, from edge to edge (even protruding beyond edges); tapering distally; folds ending before mouth area (mo), keeping smooth perioral area; inner hemipalp (ip) similar to outer hemipalp, but slightly smaller, usually placed close to anterior region of visceral mass.

Visceral mass (Fig. 24). All visceral structures white. Stomach (st) occupying most of anterior half, disposed anterior-ventrally. Digestive diverticular (dg), lying along anterior region of stomach. Gonad and genital structures occupying anterior ~half of posterior half of visceral mass, covering posterior surface of stomach. Reno-pericardial



Figures 27–30. *Eupera troglobia* anatomical drawings **27** peri-siphonal, posterior region, right view, right gill and mantle lobe removed, siphons longitudinally sectioned, visceral structures seen as in situ **28** transition between palps and esophagus, right view, with concern to cerebral ganglia **29** pedal ganglia, dorsal view **30** gross anatomy of young specimen from gill brood pouch, right view, right mantle lobe removed. Scale bars: 0.5 mm.

structures occupying posterior half of posterior half of visceral mass, up to posterior adductor muscle. Details below.

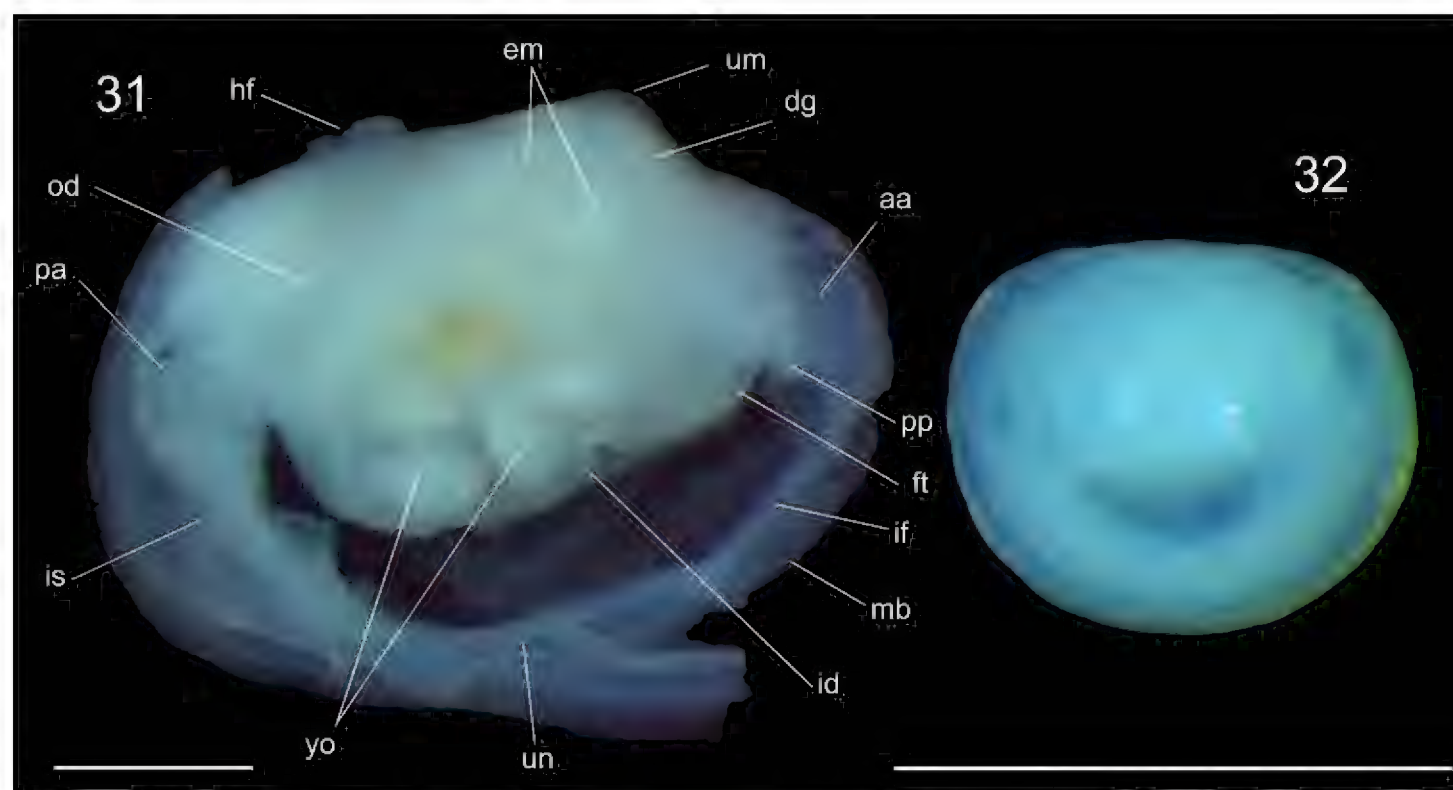
Circulatory and excretory systems (Figs 24, 27). Heart occupying anterior half of reno-pericardial area. Ventricle (ve) large, as dorsal structure, totally surrounding intestine; wall thick. Anterior and posterior aortas initially running attached to adjacent intestine. Pair of auricles (au) connected to anterior region of ventricle, each one conic, running towards ventral and lateral; connecting to central region of gills. Kidneys (ki) as posterior half of reno-pericardial volume, connected anterior and dorsal surface of posterior adductor muscle; anterior region hollow, as urinary chamber; nephropore (ur) as single, small slit located in ventro-anterior surface of supra-branchial chamber; posterior region mostly filled by white, solid renal tissue.

Digestive system (Fig. 24). Palps (pp) and mouth (mo) (Fig. 25) described above (pallial cavity). Esophagus (es) simple, narrow, running along ~20% of shell length from posterior region of anterior adductor muscle towards posterior and dorsal, inserting in anterior surface of stomach between its middle and dorsal thirds. Stomach (st) large, dorsally rounded, ventrally tapering towards ventral and anterior up to anterior region of foot base. No clear separation between intestine and style sac (ss). Duct to digestive diverticula (dd) located in center of both gastric lateral walls. Stomach inner surface simple, lacking chambers and large folds; gastric shield thin, located in postero-

dorsal region. Intestine (in) subtly running posteriorly and dorsally after style sac end, flanking dorsal surface of foot base; short zigzag only in its middle level; intestinal length slightly larger than shell length; in pericardial region crossing directly, gradually directing ventrally and posteriorly up to posterior side of posterior adductor muscle, initially immersed in pallial edge tissue; after short distance running on supra-anal chamber (Fig. 27). Anus (an) simple, sessile, located between posterior and ventral surface of posterior adductor muscle; large anal papilla in middle of anal dorsal edge (Fig. 27: an).

Reproductive system (Fig. 24). Gonad white, solid, small, mostly located in lateral regions of stomach. Large hollow brood pouch as posterior 2/3 of genital structures, full of large embryos (em); brood pouch tapering towards ventral and posterior, opening in both sides in small orifice (ap) located in middle level of suprabranchial chamber.

Central nervous system (Figs 24, 27–29). Pair of cerebral ganglia (ce) located in region dorsal to mouth, each one $\sim 1/20$ of anterior adductor muscle size. Each ganglion (Fig. 28: ce) elliptical, $\sim$ twice longer than wide; cerebral commissure (cc) wide, as long as each ganglion. Pair of pedal ganglia (Fig. 29: pg) located in middle level of pedal base; both totally fused with each other, forming spheric mass; pair of statocysts (sy) very small, located in posterior side of pedal ganglia; both pedal ganglia slightly larger than one cerebral ganglion. Pair of visceral ganglia (Fig. 27: vg) located slightly anterior to posterior adductor muscle central side; each ganglion fusiform, ~ 3 times longer than wide, located very close to each other, with pedal commissure very short; each visceral ganglion slightly larger than each cerebral ganglion; posteriorly single large nerve running towards posterior, ventrally to posterior adductor muscle (nv).



Figures 31–32. *Eupera troglobia* development, paratype MZSP 153866 pregnant specimen (2–3) **31** whole right view, shell and part of right mantle mole removed, young specimens (yo) seen in marsupium of inner demibranch by translucency **32** young specimen extracted from marsupium, right view. Scale bars: 1 mm.

Development. Large embryos found in gonadal brood pouch located inside visceral mass (Fig. 24: em). Embryos coming out by genital orifice (Fig. 24: ap), located in middle level of inner demibranchs. Both inner demibranchs serving as external branchial brood pouches (Fig. 31: yo), becoming full of young specimens (yo) in their internal area between both lamellae. Young specimens with prodissoconch of ~0.2 mm (Fig. 20), growing up to teleoconch becoming ~3-times larger than prodissoconch (Figs 19, 32), with ~0.7 mm. Prodissoconch almost plane, circular, smooth (Figs 19, 20); teleoconch possessing only concentric undulations and growth lines (Figs 19, 32); valves translucent (Fig. 32). Young intra-brood pouch specimen rounded, slightly flattened, dorsal region almost straight, umbos not protruding (Figs 19, 32); shell lacking teeth in hinge and with almost no inner muscular scars (Fig. 21). Gross anatomy of these young specimens (Fig. 30) with very small adductor muscles (aa, pa), with anterior slightly more ventral; mantle lobes edges (mb) not fused with each other, lacking siphons; gill with only inner demibranch visible (id), relatively squared, possessing 7–8 transverse folds only; foot lacking visible byssal furrow.

Material examined. Types.

Measurements (in mm). *Holotype* MZSP 155717 (Figs 1–6): 4.3 by 3.5; Paratypes MZSP 153866: #7 (Figs 7–11): 2.3 by 1.9; #8 (Fig. 12): 4.5 by 3.6.

Etymology. The specific epithet refers to the troglobitic mode of life of the animal, being an adjective in the feminine nominative singular.

Habitat. Specimens of *Eupera troglobia* sp. nov. were only observed in the Casa de Pedra cave, and are possibly endemic to this cave (Fig. 33A, B). The Casa de Pedra cave comprises a cave in limestone from the Couto Magalhães Carbonatic Formation, associated with the Neoproterozoic basement of the Baixo Araguaia Supergroup, which, in addition to the limestones, presents subordinate phyllites, slates, metargillites, metarenites and quartzites (Pereira and Moraes 2012). The climate of the region is tropical, with two distinct periods: a dry season, between May and September, and a rainy season, between October and April, with a total annual rainfall around 1750 mm (Martins et al. 2002).

The cave has 1,038 meters of total length, with predominantly ellipsoidal conduits. There are few speleothems, in addition to thick allochthonous sediments on the cave floor. The cave is inserted in a limestone outcrop located close to the Lagoa da Confusão karstic lake (Figs 33C, 34A), which overflows during the rainy season, flooding part of the flood plain surrounding it. In such periods (October to April), most cave conduits are completely filled with water. On the other hand, the cave becomes dry during the dry season, as few intermittent dams are present.

A visit paid to the cave in August 2021, revealed the cave partially flooded, with most conduits inaccessible. The main entrance gallery was filled with water, which was still forming a small lake outside the cave (Fig. 34A). Reaching the deepest areas inside the cave through the main entrance was impossible in that moment, but since there is another entrance in the middle of the cave (Figs 34B, 35A), the inner portions were accessible (Fig. 35B).

Individuals of *Eupera troglobia* sp. nov. were found associated to a consolidated sediment deposit in a deeper portion of the cave (Figs 33D, 35C). Many specimens

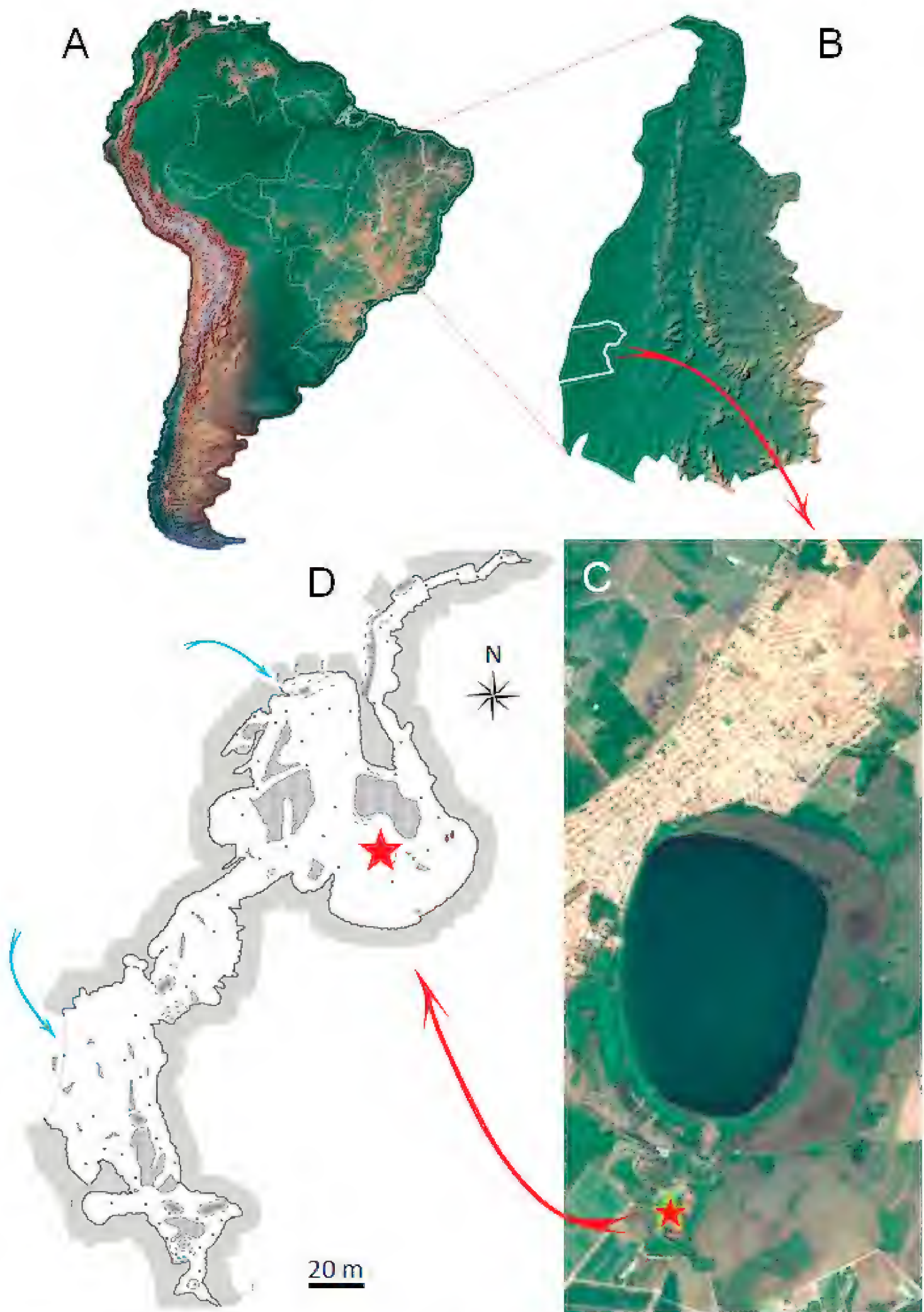


Figure 33. Location of the Casa de Pedra cave **A** South America, with Brazilian states highlighted **B** Tocantins state with Lagoa da Confusão municipality highlighted in white lines **C** aerial view from the Lagoa da Confusão region, where the urban area and the karst lake are visible; red star indicating the location of the Casa de Pedra cave **D** Casa de Pedra cave; red star indicating the location of *Eupera troglobia* sp. nov.



Figure 34. Lagoa da Confusão karst area **A** limestone outcrops close to the Lagoa da Confusão karst lake. The dark blue indicates the floodplain (not flooded in the moment of the photograph) and the dark blue indicates those flooded areas. The arrow indicates the main entrance of the Casa de Pedra cave **B** outcrop where the Casa de Pedra cave is located; 1. indicates the main entrance of the cave; 2. Upper entrance.

were adhered to the sediments already exposed to the air (Fig. 35E, F), while others were still under water (Fig. 35D, H, G). However, it is important to note that the cave was still drying up, so all specimens would be exposed to the air. Considering that the cave remains out of water during at least three months a year, the individuals do survive during all this period somehow avoiding desiccation. The only known cave-restricted clam species, all belonging to the genus *Congeria*, from caves in the Dinaric Alps, also exhibit this behavior, presenting a notable tolerance to air exposure (Jovanović et al. 2016). Interestingly, in the case of all three *Congeria* species, only part of their popula-



Figure 35. Casa de Pedra cave **A** secondary upper entrance of the cave **B** cave chamber that was partially flooded **C** area inside the cave where specimens of *E. troglobia* sp. nov. were found **D** detail of the consolidated sediment indicating the area where submerged specimens of *E. troglobia* sp. nov. were found **E** *E. troglobia* sp. nov. specimens in situ exposed to the air **F** same, detail of an air exposed specimen, with a harvestman (*Eusarcus* sp.) near it **G** in situ submerged specimens of *E. troglobia* sp. nov. **H** location of a submerged specimen of *E. troglobia* sp. nov.

tions becomes exposed during dry periods, and most part of the population remains underwater in such periods. Furthermore, even considering that *Congerina* specimens are able to tolerate air exposure for periods as 2 months, some individuals were observed still active, with their shells open and inhalant and exhalant syphons extruded (Jovanović et al. 2016). In the case of *E. troglobia* sp. nov. the single visit paid to the

cave does not make it possible to form any hypothesis regarding the individuals' behavior along the air exposure (e.g., whether they remain active or not). Accordingly, it is highly recommendable that further studies investigate the biology and life cycle of this species. It is worth mentioning that Silva (2006), in her report from the present cave, mentioned the presence of clams also associated to root masses pending from the cave ceiling during their survey (in the dry period). In that case, all specimens were also exposed to the air, and there were only few small ponds inside the cave, apparently devoid of clams.

During the clam sampling in August 2021, some hydrochemical parameters were evaluated, both inside the cave and in the epigean lake (Lagoa da Confusão lake), which floods to the cave during rainy periods. The parameters inside the cave were quite distinct from those from the external lake: cave waters: temperature: 23.6 °C; pH 6.17; conductivity: 0.124 mS/cm; dissolved oxygen: 0.92 mg/L; TDS (total dissolved solids): 0.08 g/L; Salinity: 0.06‰; external lake: temperature: 28.1 °C; pH 7.14; conductivity: 0.017 mS/cm; dissolved oxygen: 10.35 mg/L; TDS (total dissolved solids): 0.011 g/L; Salinity: 0.01‰. It is noticeable the differences in temperature (lower inside the cave), pH (lower inside the cave), conductivity (higher inside the cave) and dissolved oxygen (much lower inside the cave). This certainly demonstrates that the species is not only able to survive in conditions quite distinct from those observed in surface waters, but also probably tolerates high levels of variation in hydrochemical parameters along the year, considering that the cave water originates from the lake flooding.

Finally, it is also worth mentioning the number of embryos found in *E. troglobia*. Although in the literature, it is usual to find the term “embryo” referring to both the true embryos and the young, such stages are, in fact, distinct. True embryos (still in ontogenetic development) are those individuals found in the visceral marsupium. Those found in the inner demibranchs are called “young”, as they are already formed and the shell shows growth lines. In *E. troglobia*, there are a maximum of 10 young in each gill (~20 in total) and another 5–6 embryos in the visceral marsupium (on each side – 10–12 in total). Hence, the species presents around 30 immatures (considering both embryos and young). In the consulted literature, only the young specimens inside gills are considered. The other already studied *Eupera* species (all epigean), presented a considerably larger reported number. As an example, *E. platensis* had between 22 and 66 young specimens in gills (Ituarte 1988); *E. cubensis* between 25 and 35 (Heard 1964) and *E. klappenbachii* had between 24 and 62 (Mansur and Veitenheimer 1975). Most cave-restricted species from several distinct groups usually have k-strategies (Howarth 1983; Bellés 1992), due to the relatively stable environments that subterranean habitats usually present. Among the reproductive adaptations related to such strategy, there are a reduced number of offspring, increased offspring body size, parental care, among others. The reduced number of embryos compared to some epigean *Eupera* species, associated to the proportional large size of the young observed in the visceral marsupium of *E. troglobia*, probably represent another adaptation to the cave environment, confirming the cave-restricted status of this species.

Discussion

Taxonomical considerations

All known South American species of *Eupera* (Simone 2006) have well-defined dark spots in the central region of the valves, contrasting with the light brown background. *E. troglobia* sp. nov. is the only species that lacks these dark spots and has a shell of uniform pale yellow color, slightly distinguishing the new species from its relatives. Besides color, outline is also useful to distinguish *E. troglobia*; it is more elongate antero-posteriorly than *E. bahiensis* (Spix in Wagner 1827); *E. iguazuensis* Ituarte, 1994; *E. klappenbachi* Mansur & Veitenheimer, 1975; *E. modioliforme* (Anton, 1837); *E. primei* Klappenbach, 1967; *E. simoni* (Jousseaume, 1889) and *E. tumida* (Clessin, 1879). On the other hand, compared to *E. doellojuradoi* Klappenbach, 1962, it is not as elongate antero-posteriorly and the anterior region is not as acuminate as that of *E. guaraniana* Ituarte, 1994. The only species that has a similar outline to *E. troglobia* is *E. platensis* Doello-Jurado, 1921, from which *E. troglobia* differs in having higher and narrower umbos, and being somewhat more laterally compressed. The hinge teeth of most species of *Eupera* are more developed than those of *E. troglobia*, especially the anterior lateral tooth is almost inconspicuous, while in the other species it is thick and prominent; the only exception is *E. doellojuradoi*, which also has narrow hinge teeth.

The anatomy of *E. troglobia* is similar to the congeneric species whose anatomy is known (Mansur and Veitenheimer 1975), especially the smallness of the palps, the arrangement of the gills and the mantle margin. The most important anatomical differences from *E. klappenbachi* are the apparently proportionally smaller size of the foot and siphons; the intestine is also much less tortuous and is located entirely posterior to the stomach, whereas part of it is anterior to the stomach in *E. klappenbachi*.

The histological arrangement of *E. platensis* was detailed by Ituarte (1977). The species is revealed to be simultaneously hermaphrodite. The production of yolky eggs and the incubation of embryos and young species was well documented. As far as possible, *E. troglobia* also has similar features. About 60% of the specimens have pregnant gills, while the remaining specimens appeared still submature. Synchronous gills brooding appears to be characteristic of the entire family (Cooley and Foighil 2000), being modified to sequential in *Sphaerium* Scopoli, 1777.

The histological arrangement of *E. platensis* was described in detail by Ituarte (1977). The species is simultaneously hermaphroditic. The production of yolky eggs and the incubation of embryos and juveniles have been well documented. As far as possible, *E. troglobia* also exhibits similar characteristics. About 60% of the specimens have pregnant gills, while the remaining specimens appeared immature. Synchronous gill brooding appears to be characteristic of the entire family (Cooley and Foighil 2000), although it was modified to sequential in *Sphaerium* Scopoli, 1777.

The anatomy of other sphaeriid genera is better known, especially those from Europe. The kidney of *Eupera* looks simpler than at least those of *Pisidium* Pfeiffer, 1821, and *Sphaerium*, which have a coiled urinary chamber (Cooley and Foighil 2000; Ituarte and Korniuschin 2006; Korniuschin and Glaubrecht 2006; Mansur et al. 2008). Pallial

musculature is also more uniform in *Eupera*, whereas in the other genera it is concentrated at specific locations on the valves. *Eupera* is also known for the absence of a developed brood sac in the gills, which is present in the other genera (Cooley and Foighil 2000).

It is difficult to determine whether *Pisidium* and *Euglesa* are troglobitic organisms because some populations are found in caves (Turbanov et al. 2016; Grego et al. 2019; Boessneck et al. 2020; Groh et al. 2020), as mentioned in the introduction. Both genera comprise possibly the smallest freshwater bivalves and are highly simplified. In addition, their interstitial life in the sediment is usually already associated with tiny, unpigmented, and fragile shells that overlap with troglobite assemblages. Certainly, further studies of these sphaeriids could yield additional surprises about the evolution of troglobitic bivalves.

Conservation issues

The Lagoa da Confusão karst area is spatially restricted, with only four small carbonate outcrops near the lake. The outcrop farthest from the lake (about 3 km in a straight line from the lake) has suffered from limestone quarrying, which has apparently led to the destruction of the largest cave occurring there. The other three outcrops near the lake, on the other hand, have been preserved, although the surrounding landscape has been greatly altered, primarily by agriculture. There are only three known caves in these outcrops, of which Casa de Pedra Cave is the largest. The Casa de Pedra cave is used by the locals for religious purposes. Every year in November, when the cave is dry, a mass for the deceased is celebrated in the main entrance chamber. During this time, visitors leave behind a lot of trash, such as candles, plastic bottles, and more. Fortunately, most visitors stay only in the entrance chamber and do not enter the deeper parts of the cave where individuals of *Euperia troglobia* sp. nov. occur. However, it is impossible to assess whether the species is affected by such activities. Considering that it is a filter-feeding species, any contaminants released by trash left behind by visitors could be a problem for the species. Other contaminants, such as pesticides and fertilizers used on surrounding agricultural plantations, are also a problem because during rainy seasons the lake's floodplain fills up, spreading these substances over large areas, including the cave. Brazilian laws on the national speleological heritage require that a management plan be prepared for each cave that is used by people (for recreational purposes, religious purposes, etc.). Therefore, it is imperative that a study is conducted in Casa de Pedra Cave to determine if the current religious use is altering or even threatening the cave fauna, particularly the unique population of *E. troglobia* sp. nov. Based on this investigation, a management plan can be created and implemented.

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